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COMPLEMENT FIXATION IN DIAGNOSIS OF TUBERCULOSIS

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Since there have been good reviews of the literature on this subject published recently,¹ it does not seem advisable at this time to review it again. Further, no effort will be made to give a detailed classification of the cases, as it is felt that in the hands of different clinicians the same set of cases would receive different classifications. Cases considered as incipient by some observers would be considered as moderately advanced by others and vice versa. Varying degrees of importance are placed on certain symptoms or physical signs by different observers, and the enthusiastic observer might find what he considers evidence of tuberculosis in a case which on more thorough and more extended observation proves to be nontuberculous. The cases reported in this article have been grouped as follows: active cases, inactive cases and nontuberculous cases. It is realized that in some cases the differentiation between activity and inactivity might be impossible. However, the clinical examinations of these cases were made by well qualified, competent and conservative clinicians, and it is felt that the classification is as near correct as can be obtained.

The complement fixation tests were made independently of the clinical examinations and no reference was made to the clinical records until the series of fixation tests had been completed.

TECHNIC

Serum.—The serum was removed from the clot as soon as possible and inactivated at 56 C. for 30 minutes; 0.1 c.c. of the undiluted serum was used in the test and the same amount was used as a control for anticomplementary action.

Antigen.—Petroff's Antigen² 2 was used, 250 mgm. of dried, pulverized tubercle bacilli were extracted with 50 c.c. of methyl alcohol at 38 C. for from 5-10 days, when it was well corked and set aside in a dark place at room temperature. After several weeks the supernatant fluid was pipetted off and was ready for use. The container should be sealed or well corked. When used it is diluted with the necessary amount of salt solution as determined by titration.

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¹ Stoll, H. F., and Newman L.: Jour. Am. Med. Assn. 1919, 72, p. 1403; Von Wedel, H. J.: Jour. of Immunology, 1918, 3, p. 351; Moon, V. H.: Jour. Am. Med. Assn., 1918, 71, p. 1127; Stivelman, B.: Am. Rev. Tuberc., 1918, 546, p. 550.

² Am. Rev. Tuberc., 1917, 1, p. 35.

Using 0.1 cc positive serum, 0.01 cc antigen gives partial fixation; 0.05 cc gives fairly complete fixation; 0.1 cc gives complete fixation. With negative serum it is not anticomplementary until 0.5 cc of the antigen is used. The tests were made by using 0.1 cc of antigen. Before setting up tests, the antigen was titrated each day to determine fixing power with positive serum, and anti-complementary limit.

Complement.—The pooled serum from 4 to 6 guinea-pigs was used in a 1:10 dilution. The complement was titrated, using 3 units of amboceptor and 0.1 cc of 5% sheep cell suspension, 0.05 cc of pooled negative serums (10 serums containing no natural amboceptor) with 0.1 cc of antigen in the front row of tubes. The amount of complement to be used in the tests is indicated by the smallest amount necessary for complete hemolysis.

Hemolytic System.—One-tenth cc of 5% washed sheep corpuscles is used with 0.2 cc of diluted amboceptor containing 3 standard units.

Controls.—Known positive and negative serums were used as controls each day.

The titrations of complement and amboceptor are incubated in water bath at 37.5 for 1 hour and the fixation tests in icebox for 2 hours. Then in water bath for 30 minutes and after addition of sheep cells and amboceptor for 1 hour in the water bath. The tests are read immediately after last incubation.

In a certain number of cases both water bath fixation at 37 C. and icebox fixation at about 10 C. were used with the same serums. The results obtained would indicate that the icebox method gives better results in that more positives were obtained and the fixation in doubtful water bath reactions became more complete in the icebox method.

Tests were made on the serums of 186 patients, 103 being active cases and 51 inactive cases of pulmonary tuberculosis; one was a case of tuberculosis of the epididymis (test made some time after operation) and 31 were nontuberculous cases.

Of the 103 active cases, 15 gave ++ reactions, 1 gave a + reaction, 5 gave ± reactions, and 83 gave negative reactions. Considering the ++ and + reactions as positive, only 16, or 15.53%, of the total number of active cases gave positive complement fixation tests.

Of the inactive cases, 50 gave negative reactions and one gave a ++ fixation test for tuberculosis and also a ++ Wassermann reaction.

Of the nontuberculous cases, 29 gave negative reactions and one gave a ++ fixation test for tuberculosis and also a ++ Wassermann reaction.

Sputum examinations were made in all cases. The antiformin concentration method was used. From one to eight sputum examinations were made in each case. Of the 103 active cases, 10 gave positive sputum and positive complement fixation tests, 6 gave negative sputum and positive fixation tests, 41 gave positive sputum and negative fixation tests, and 46 gave negative sputum and negative fixation tests.

DISCUSSION

A laboratory test to be of value to the clinician must be either of diagnostic or prognostic aid. The degree of activity in the 103 cases varied considerably, and in the 16 cases giving positive complement fixation tests the degree of activity varied so markedly that the fixation test cannot be considered of any prognostic value.

The test is of very little, if any, value as a diagnostic aid, at least in the type of cases examined. Of the 16 cases which gave positive complement fixation tests, 10 gave positive sputum at the time of or before the fixation test was made. So in only 6 cases of the entire series of active and inactive cases can the complement fixation test be said to have been of any diagnostic aid. Also without the laboratory tests the diagnosis could be easily made in all these cases by the clinician with the aid of the roentgen-ray laboratory. It will be noted that approximately 50% of the active cases had tubercle bacilli in the sputum, and no doubt further sputum examinations in the other cases would increase this percentage.

Since the inactive cases, except the one which gave a positive Wassermann test, gave negative fixation tests for tuberculosis, it might be considered that the test is of some importance in distinguishing active from inactive cases. However, when one stops to consider the large number of active cases that gave negative reactions it does not seem that the test can be considered of any practical value in differentiating active from inactive cases. The same thing can be said relative to the value of a negative reaction in excluding the presence of tuberculosis in a patient.

Of course it is realized that the system of the fixation test used in the examination of these cases might not be the ideal one for this kind of work, and also the antigen used may not be the best.

Another question to be decided in connection with this test is whether or not it is a specific reaction. In all cases in this article a Wassermann test was made at the same time as the fixation test for tuberculosis. Of the cases giving positive fixation for tuberculosis 3 cases also gave positive Wassermann tests. In 2 of the 3 cases the patients denied all history of syphilitic infection and there was nothing to indicate the presence of such infection.

In two of the active cases giving negative fixation for tuberculosis, positive Wassermann reactions were obtained. In neither case was there any history nor evidence of any syphilitic infection.

TABLE 1
WASSERMANN AND COMPLEMENT FIXATION TESTS

Serum	Syphilis Showing Strength of Reaction						Tuber- culosis, 0.1 c c	Gono- coccus, 0.1 c c	Glanders, 0.1 c c	Strepto- coccus, 0.1 c c
	0.05 c c	0.025 c c	0.0126 c c	0.0062 c c	0.0031 c c	0.0015 c c				
1	++	0	0	0	0	0		0	0	0
2	++	0	0	0	0	0		0	0	0
3	++	0	0	0	0	0		0	0	0
4	++	0	0	0	0	0		0	0	0
5	++	0	0	0	0	0		0	0	0
6	++	++	++	++	++	±	++	0	0	0
7	++	0	0	0	0	0		0	0	0
8	++	0	0	0	0	0		0	0	0
9	++	0	0	0	0	0		0	0	0
10	++	0	0	0	0	0		0	0	0
11	++	0	0	0	0	0		0	0	0
12	++	0	0	0	0	0		0	0	0
13	++	0	0	0	0	0		0	0	0
14	++	0	0	0	0	0		0	0	0
15	++	0	0	0	0	0		0	0	0
16	++	++	++	++	++	±	++	0	0	0
17	++	++	++	++	+			0	0	0
18	++	++						0	0	0
19	++	++	++	++			++	0	0	0
20	++	++	++	++	++			0	0	0
21	++							0	0	0
22	++									
23	++	++	++	++				0	0	0
24	++	++						0	0	0
25	++	++	++	±				0	0	0
26	++	++	++	++				0	0	0
27	++						+	++	0	0
28	++	++	++	++	+			++	0	0
29	++	±						++	0	0
30	++	++	++	+				+	0	0
31	++	++	+							
32	++	+								
33	++						++			
34	++	++	++	++	±		++	+		0
35	++	++	±							0
36	++	++	±				++	++		
37	++								0	0
38	++	++	+						0	0
39	++	++						0	0	0
40	++	++						++	0	0
41	++	++					+		0	0
42	++	+						±	0	0
43	++	++	+						0	0
44	++	++	+	±				++		
45	++	++					++	++		
46	++	++	++	+				+		
47	++	++	++	++					0	0
48	++	++	++	+				+		
49	++	++						++		
50	++	++	±					0		
51	++	++	+	±				0		
52	++	++	++	+	±			0		
53	++	++	++	++	±			0		
54	++	++	++	++	+		+	0		
55	++	++	±					0		
56	++	++	+				+	0		
57	++	++	++	+			++	0		
58	++	++	++	±			++	0		
59	++	++	++	++			++	0		
60	++	++	++	±				0		
61	++	++	+				+	0		
62	++	++	++	+	±			0		
63	++	++	++	+			++	0		
64	++	++	++	++	±		++	0	+	
65	++	++	++	+			++	0		
66	++	+	±				+	0	0	0
67	++	++	++	+	±		++	0		
68	++	+					++	0		
69	++	++	++				+	0		

TABLE 1—*Continued*
WASSERMANN AND COMPLEMENT FIXATION TESTS

Serum	Syphilis Showing Strength of Reaction						Tuberculosis, 0.1 c c	Gonococcus, 0.1 c c	Glanders, 0.1 c c	Streptococcus, 0.1 c c
	0.05 c c	0.025 c c	0.0126 c c	0.0062 c c	0.0031 c c	0.0015 c c				
70	++	++	++	++	+	±	++	0		
71	++	++	+	++	+		+	0		
72	++	+					++	0		
73	++	++	++	+	±		++	0		
74	++	++	++	±				0		
75	++	++						0		
76	++						+	0		
77	++	++	++	++	++	++	++	0		
78	++	++	++	+	±		++	0		
79	++	+	±				+	0	0	0
80	++	++	±				+	0		
81	++	+						0	0	0
82	++	++	++	++	+	±	+	0		
83	++	++	++	+			++	0	0	0
84	++	++					++	0		
85	++	++	++				+	0	+	
86	++	++	++	++	++	++	++	0		
87	++	++	++	+				0	0	c
88	++	++	+	±				0		
89	++	++	++	++	++	+	++	0		
90	++	++	++	++	+	+	++	0	0	c
91	++	++	++	++	+	+	+	0	0	0
92	++	++	++	++	+	±		0		
93	++	++	++	++	++	+	++	0		
94	++	++	++					0		
95	++	+						0		
96	++	++	++	++	+		++	0		
97	++	+					+	0		
98	++	++	±				+	0		
99	++	++	++	++	++	++	++	0		
100	++	++	++	++	++	+	+	0		

Of the inactive cases, one gave ++ reactions in both tests. This patient gave a history of syphilitic infection, but nothing to indicate the presence of tuberculosis. That a patient might have tuberculosis and syphilis at the same time must be recognized, but that all these cases giving both positive fixation for tuberculosis and syphilis had both tuberculosis and syphilis seems very questionable.

It is still believed by some workers that a few cases of tuberculosis give a positive Wassermann reaction without any evidence or history of syphilitic infection. That the Wassermann test is not specific is recognized by serologists. While it is generally supposed that bacterial complement fixation tests are specific, this work suggests the possibility that they are nonspecific. An attempt was therefore made to determine whether serum that gave a positive complement fixation for syphilis would also produce a positive complement fixation with various bacterial antigens. The table presents the results obtained.

Complement fixation tests for tuberculosis (as described) were made in 100 patients who gave a ++ complement fixation for syphilis; 45 gave positive fixation tests for tuberculosis. Fixation tests using gonococcus antigen were made in 28 with positive results in 12. Fifty-nine were tested with glanders antigen with weak + reactions in 3; 55 were tested with streptococcus antigen with no positive reactions. In 86 the serum was titrated to determine the strength of complement fixation for syphilis, and from these titrations the following summary is compiled.

TABLE 2
COMPLEMENT FIXATION FOR SYPHILIS

Dilution of Serum	0.05 c c	0.025 c c	0.0125 c c	0.0062 c c	0.0031 c c	0.0015 c c
Positive tubercle fixation.....	3	11	6	10	6	8
Negative tubercle fixation.....	4	13	10	12	3	0
Per cent. positive.....	42.8	45.8	37.5	45.4	66.6	100

In the cases giving positive fixation for syphilis in serum dilution from 0.05 c c to 0.0062 c c there is no marked difference in the percentage of positive cross fixation with tubercle antigen in each dilution, but the percentage of positives increases in the cases giving positive fixation for syphilis in dilutions higher than 0.0062 c c, even giving 100% positive in the dilution of 0.0015 c.c. This would indicate that the greater the fixing properties of the serum with syphilitic antigen, the greater the possibility of cross fixation with tubercle antigen. To determine whether or not only syphilitic serum would give cross fixation with tubercle antigen, 100 serums giving negative fixation for syphilis were tested for fixation with tubercle antigen. Of this number, 17 gave positive complement fixation for tuberculosis, none of which gave any history or evidence of tuberculosis, and in only two was there a history of syphilis (two old treated cases). Naturally the question would arise as to the possibility of double infections explaining the cross fixation of syphilitic serums with tubercle and gonococcus antigens.

In 15 of the cases giving cross fixation with tubercle antigen and in 4 giving cross fixation with gonococcus antigen there was no history obtainable. In only 2 of the cases was there a history of tuber-

culosis, both being cases of active chronic pulmonary tuberculosis. Of the 8 cases giving cross fixation with gonococcus antigen, and in which the history obtained is dependable, none gave a history of recent gonococcus infection, and only two gave a history of such infection, one in 1910, the other in 1917.

It can hardly be conceived that approximately 45% of all syphilitic individuals also have tuberculosis without any signs or indications of the latter disease.

It is recognized that a large number of individuals at some time during life become infected with tubercle bacilli, but the disease is arrested in some and cured in the majority. Granting that all patients giving cross fixation have had tuberculosis at some time during life, it does not seem possible that complement fixing bodies would remain for any length of time after such cases are arrested or cured. The positive Wassermann in a syphilitic is considered indicative of activity of the disease and that such a case should receive more treatment. From the work done, it is not believed this rule can be applied to complement fixation tests with bacterial antigens.

Apparently, the serums of a large number of syphilitics and of some normal individuals contain complement fixing bodies in the presence of tubercle and gonococcus antigens. What these fixing bodies are is not known. Possibly they are of a protein nature, and when in sufficient quantity combine with lipoids in the antigen to produce fixation of complement.

An idea is advanced that the greater the amount of lipoidal substances contained in an antigen, the less specific such an antigen is, since in the series of tests reported in this article it is noted that cross fixations are more numerous with tubercle and gonococcus antigens than with glanders and streptococcus antigens, the tubercle bacilli and gonococci containing more lipoidal substances than either the glanders bacilli or streptococci.

CONCLUSIONS

The complement fixation test for tuberculosis as described in this article is of no value as a diagnostic or prognostic aid.

The complement fixation test for tuberculosis with alcoholic extract of tubercle bacilli as antigen, is not specific.

Not all complement fixation tests with bacterial antigens are specific.

A large percentage of serums giving a positive Wassermann give fixation with tubercle and gonococcus antigens.

A certain number of individuals not infected with tuberculosis or gonorrhea will give positive fixation tests with one or both of the corresponding antigens.